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Skeletal muscle UCP2 and UCP3 expression in trained and untrained male subjects

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OBJECTIVE: The new uncoupling proteins, UCP2 and UCP3, are thought to play a role in energy efficiency in humans. Endurance training has been suggested to have effects on resting metabolic rate and energy efficiency. We therefore determined UCP2 and UCP3 mRNA levels in skeletal muscle of trained and untrained male subjects.
METHODS: Using reverse transcription-polymerase chain reaction (RT-PCR), expression of UCP2, UCP3L and UCP3S mRNA were measured in muscle biopsies from the quadriceps femoris in eight trained (23.9±1.6 y; 70.6±3.1 kg; 14±3% body fat; maximal power output (W_{max}): 5.6±0.4 W/kg; mean±s.d.) and 10 lean, untrained (22.1±2.9 y; 72.0±7.9 kg; 18±4% body fat; W_{max}: 3.9±0.4 W/kg; mean±s.d.) subjects. In six of the trained subjects, UCP2 and UCP3 mRNA were measured before and after an exercise bout to exhaustion. To correct for differences in mitochondrial content, levels of UCP2 and UCP3 mRNA were expressed relative to cytochrome-b, a marker of mitochondrial content.
RESULTS: Acute exercise had no effect on the expression of UCP3L or UCP3S, but in five out of six subjects UCP2 expression decreased after exercise, although the difference was not statistically significant ($P=0.11$). Trained subjects had significantly reduced mRNA levels of UCP3L ($P=0.028$) and UCP3S ($P=0.031$). VO_{2max} expressed per kg of fat-free mass was negatively correlated with UCP3L ($r=-0.61$, $P=0.009$) and UCP3S ($r=-0.52$, $P=0.028$). Mechanical efficiency correlated negatively with UCP3L ($r=-0.56$, $P=0.019$), UCP3S ($r=-0.47$, $P=0.048$) and tended to correlate with UCP2 ($r=-0.46$, $P=0.06$).
CONCLUSION: The lower levels of UCP3 mRNA in trained subjects and the inverse relationship of UCP3 expression and mechanical efficiency suggest that exercise training produces an adaptive physiological response in skeletal muscle improving mechanical efficiency.

Keywords: UCP gene expression; energy expenditure; endurance training

Introduction

In rodents, brown adipose tissue plays an important role in thermogenesis and energy balance, via the activation of an uncoupling protein gene, UCP1. This gene encodes for a mitochondrial protein carrier which uncouples respiration from ATP production and stimulates heat production.[1] UCP1 is only expressed in brown adipose tissue, which is scarce in adult humans and is not thought to play a major role in energy balance. Recently, two new uncoupling proteins, UCP2[2,3] and UCP3,[4,5] were discovered. UCP2 and UCP3 have approximately 55% amino acid identity with UCP1 and have been shown to have uncoupling activity.[2,6] UCP2 is expressed in many tissues, whereas UCP3 is mainly expressed in skeletal muscle.[4,5] *UCP2* was mapped to chromosome 11q13[2] and *UCP3* is only 8 kb away from UCP2.[7] In the Quebec Family study, resting metabolic rate (RMR) was genetically linked to DNA microsatellite markers in the close vicinity of *UCP2/UCP3*.[8] Recently, we found an association between polymorphisms in UCP2 and sleeping metabolic rate in Pima Indians.[9] Furthermore, we found a positive correlation between sleeping metabolic rate and UCP3 mRNA levels in Pima Indians.[10] Taken together, these results indicate that the new uncoupling proteins may be important determinants of energy expenditure in humans.

The effect of endurance training on energy metabolism has been extensively studied but results are quite controversial. Some studies have shown an increase in resting metabolic rate, adjusted for fat-free mass and fat mass, in response to training,[11,12] whereas others did not.[13–15] Also, endurance training has been proposed to decrease the thermic effect of food.[16] Furthermore, an increase in gross energy efficiency in response to training has been reported.[17,18] In the present study we measured mRNA levels of UCP2 and the different isoforms of *UCP3*, the long (UCP3L) and short form (*UCP3S*) of *UCP3*, in two groups of untrained and trained subjects. We hypothesized that endurance training results in decreased UCP2 and UCP3 levels, making endurance trained athletes more energy efficient.

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Methods

Subjects

Eighteen lean male volunteers were recruited: 10 were untrained and eight were highly trained young individuals. All subjects had to be in good health, were non-smokers and had a body mass index (BMI) between 18–25 kg/m^2. Subjects who had never participated in competitive endurance sports and had less than 3 h of activity per week for the last 3 y were included in the untrained group. Subjects who were regularly involved in competitive endurance sports for at least the past 3 y and had more than 15 h per week of endurance training were included in the trained group. Body density was determined by underwater weighing, with simultaneous measurement of lung volume with the helium dilution technique using a spirometer (Volugraph 2000, Mijnhardt). Percent body fat was calculated using the equations of Siri.[19] Subjects' characteristics are given in Table 1. Trained and untrained subjects had similar body weight, BMI and fat-free mass (FFM). The study was approved by the Ethical Committee of the Maastricht University and all subjects gave their written informed consent.

Exercise testing

Each subject performed an incremental exercise test to exhaustion on an electronically braked cycle ergometer (Lode Excalibur, Groningen, The Netherlands) to determine maximal power output (W_{max}) and maximal oxygen consumption ($\dot{V}O_{2max}$). Exercise was performed until voluntary exhaustion or until the subject could no longer maintain a pedalling rate of $\geq$ 60 rpm. Subjects started at 100 W for 5 min. Thereafter, work load was increased by 50 W every 2.5 min. When subjects were approaching exhaustion, as indicated by heart rate and subjective scoring, the increment was reduced to 25 W. In practice, this meant that the last one to three load increments were 25 W. Heart rate was measured continuously using a Polar Sport tester (Kempele, Finland). W_{max} was calculated from:

$$W_{max} = W_{out} + (t/150) \times \partial W$$

Table 1 Subjects' characteristics. Values are expressed as mean ± s.e.m.

Group	*Trained*	*Untrained*
Age (y)	23.9 ± 0.6	22.1 ± 0.9
Weight (kg)	70.6 ± 1.1	72.0 ± 2.5
Height (m)	1.82 ± 0.02	1.80 ± 0.01
BMI (kg/m^2)	21.3 ± 0.6	22.3 ± 0.8
% body fat	14 ± 1	18 ± 1
Fat-free mass	60.5 ± 0.5	59.1 ± 1.8
$\dot{V}O_{2max}$ (ml/min/kg)	66.9 ± 2.6*	51.5 ± 1.5
W_{max} (W)	396 ± 8*	277 ± 9
W_{max}/kg (W)	5.62 ± 0.16*	3.87 ± 0.12

BMI = body mass index; $\dot{V}O_{2max}$ = maximal oxygen consumption; W_{max} maximal power output.
*$P <$ 0.0001 compared to untrained.

in which W_{out} is the highest workload completed by the subject, t is the time (in s) performed on the last workload and ∂W is the final uncompleted load increment.[20] Oxygen consumption and carbon dioxide production were measured using open circuit spirometry (Oxycon-ß Mijnhard, The Netherlands).

At least 7 d after the determination of W_{max}, mechanical efficiency was determined as follows: subjects cycled for 15 min at 30, 45 and 60% of W_{max} with a fixed pedalling rate of 80 rpm. Oxygen consumption and carbon dioxide production were measured using open circuit spirometry (Oxycon-ß Mijnhard, The Netherlands) and energy expenditure was calculated as described previously.[21] Mechanical efficiency was regarded as the slope of the regression between energy expenditure and workload.

In order to measure UCP2/3 mRNA expression before and after exercise, six of the trained subjects performed intermittent exercise bouts to exhaustion, as part of another study. After a warm-up at 50% of their W_{max} for 5 min, subjects cycled 2 min on 90% of W_{max} followed by 2 min on 50% of W_{max}. This was repeated until subjects were no longer able to perform the high intensity exercise. The maximal intensity was then lowered to 80% of W_{max}. Again, when this intensity could no longer be maintained, the maximal intensity was decreased to 70% of W_{max}. The test was ended after exhaustion. Subjects were allowed to consume water during exercise. Heart rate was measured continuously with a polar sport tester.

Basal metabolic rate and blood sampling

After an overnight fast, subjects came to the laboratory at 8 a.m. Instructions were given to refrain from any exercise on the previous day. Subjects rested on a bed for 40 min and oxygen consumption and carbon dioxide production were measured using a open circuit ventilated hood system. Metabolic rate was averaged over the last 20 min, when subjects were in a steady state. Before the measurement of the basal metabolic rate, 20 ml of venous blood was taken; 10 ml for plasma in tubes containing EDTA and 10 ml in vacutainers for serum. Plasma FFA were determined using a kit (Wako chemicals, Neuss, Germany), insulin (Kabi Pharmacia) and leptin (Linco Research, St. Charles, USA.) by RIA. Serum T3 was determined using DPC immulite T3.

Muscle biopsy and RNA analysis

After measurement of resting metabolic rate, a percutaneous muscle biopsy was taken from the vastus lateralis. After local anaesthesia, a 5 mm diameter side cutting needle was passed through a 7 mm skin incision. The muscle biopsy was immediately frozen in liquid nitrogen and stored at $-$ 70°C until assayed. Muscle specimens were homogenized and total RNA was isolated using Trizol kit (Gibco-BRL, Gaithersburg, MD). Oligo-dT-primed cDNAs were synthesized from 2 µg of total RNA in a 20 µl volume using SuperScript cDNA synthesis kit from Gibco

BRL (Gaithersburg, MD). Quantitative comparisons were performed by reverse transcription Polymer chain reaction (RT-PCR), with 2 µl of first-strand cDNA in 25 µl reactions containing 800 µM dNTP, 200 nM primers, 1 U Taq DNA polymerase (GibcoBRL) and 1× PCR buffer containing 1.5 mM $MgCl_2$. All primer combinations were designed to span at least one intron to avoid co-amplification of genomic DNA which may contaminate the RNA preparation. The primers used for amplifying cDNA fragments are shown in Table 2. During PCR (94°C for 1 min, 57°C for 45 s and 72°C for 2 min), aliquots (5 µl) were taken from each tube every four cycles following 22 cycles to determine whether the amplification was in the linear phase for each product. The products were resolved on a 1% agarose gel containing 1µl ethidium bromide which was photographed using Polaroid 665 film (Cambridge, MA), and the relative concentration of PCR products was measured by scanning densitometry (BioImage version 3.3, Sun SparcStation 5, Ann Arbor, MI). Each experiment was performed in triplicate and the mean value was calculated for analysis. Levels of mRNA were expressed as the ratio of signal intensity for the target genes relative to β-actin (that is, UCP2/β-actin). β-actin is often used as an internal standard and expression is not influenced by training.[22] To correct for training-induced differences in mitochondrial content, mRNA levels were also expressed relative to cytochrome-b (that is, UCP2/cytochrome-b). In response to training, the expression of mitochondrial mRNAs is increased in direct proportion to total mitochondrial content.[23] All ratios were corrected for the size of the products.

Statistical analysis

Differences between trained and untrained subjects were tested using unpaired *t*-tests. The effect of exercise on mRNA levels was tested using paired *t*-tests. Pearson correlation coefficients were calculated to determine the relationship between selected variables. Data are expressed as means ± s.e.m. and *P*-values < 0.05 are considered significant.

Results

Effect of acute exercise

Six subjects performed intermittent bouts of exercise to exhaustion. Mean exercise time was 83.7 ± 7.8 min. There was no significant difference in UCP2 expression before and after the exercise bout ($P = 0.11$, Figure 1). However, in five out of six subjects, UCP2 expression decreased in response to exercise. UCP3L and UCP3S did not change after exercise (Figure 1).

Effect of endurance training

In the total group of 18 subjects, the expression of UCP3L correlated with the expression of UCP3S ($r = 0.77$, $P < 0.001$).

The mRNA levels of UCP3L, UCP3S and UCP2 were not significantly different between trained and untrained subjects. However, when expressed relative to cytochrome-b, UCP3L ($P = 0.028$) and UCP3S ($P = 0.031$) were significantly lower in trained compared to untrained subjects (Figure 2). UCP2 expression relative to cytochrome-b tended to be lower in trained subjects *vs* untrained ($P = 0.09$, Figure 2). In the remainder of the results, UCP2/UCP3 mRNA values are expressed relative to cytochrome-b.

The expression of cytochrome-b was positively correlated with W_{max} expressed per kg FFM ($r = 0.49$, $P = 0.04$) and $\dot{V}O_{2max}$ expressed per kg FFM ($r = 0.57$, $P = 0.014$). UCP3L ($r = -0.61$, $P = 0.01$), UCP3S ($r = -0.49$, $P = 0.042$) and UCP2 ($r = -0.48$, $P = 0.051$) correlated negatively with W_{max} expressed per kg FFM. Multiple regression revealed that the correlation between UCP3L and W_{max} expressed per kg FFM persisted when corrected for cytochrome-b expression ($P = 0.04$).

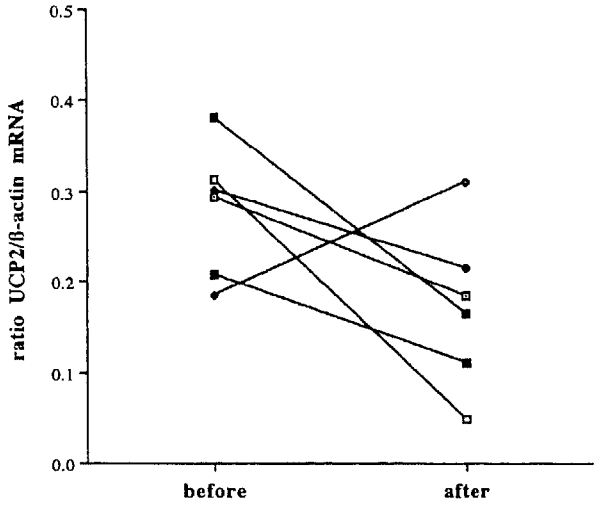
Similarly, UCP3L ($r = -0.61$, $P = 0.009$), UCP3S ($r = -0.52$, $P = 0.028$) and UCP2 ($r = -0.44$, $P = 0.08$) correlated negatively with $\dot{V}O_{2max}$ expressed per kg FFM. Again, multiple regression analysis revealed that the correlation between UCP3L and W_{max} persisted after correction for cytochrome-b expression ($P = 0.039$).

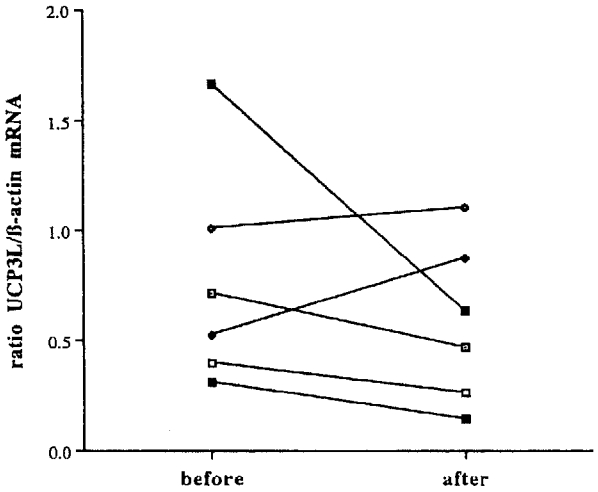
UCP3L ($r = -0.56$, $P = 0.019$, figure 3) and UCP3S ($r = -0.47$, $P = 0.048$) were negatively correlated with mechanical efficiency, but this correlation did not reach statistical significance for UCP2 ($r = -0.46$, $P = 0.06$). The expression of cytochrome-b was not correlated with mechanical efficiency, so the correlation between UCP3 and mechanical efficiency was independent of mitochondrial content (cytochrome-b expression).

Resting metabolic rate, adjusted for fat-free mass and fat mass, was not significantly different between trained and untrained subjects and did not correlate with mRNA levels of UCP3L, UCP3S or UCP2.

Table 2 Sequences of polymerase chain reaction primers

Gene	*Sense primer (5'-3')*	*Antisense primer (5'-3')*	*Size of cDNA, bp*
UCP2	catctcctgggacgtag	atcaggtcagcagcaggagag	964
UCP3L	aggactatggttggactgaa	cattcttaactggtttcggacac	993
UCP3S	aggactatggttggactgaa	gttctctgggagggagtgc	868
β-actin	actgactacctcatgaagat	cgtcatactcctgcttgctgat	535
cytochrome-b	ggttctggaataagaatatagg	gacaacacagtaagaaccagg	367





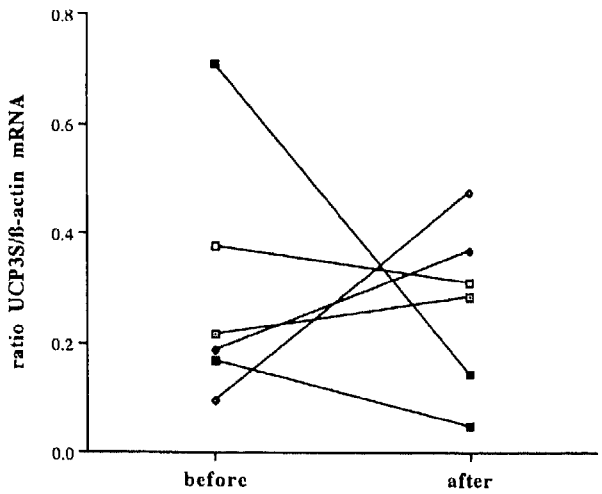
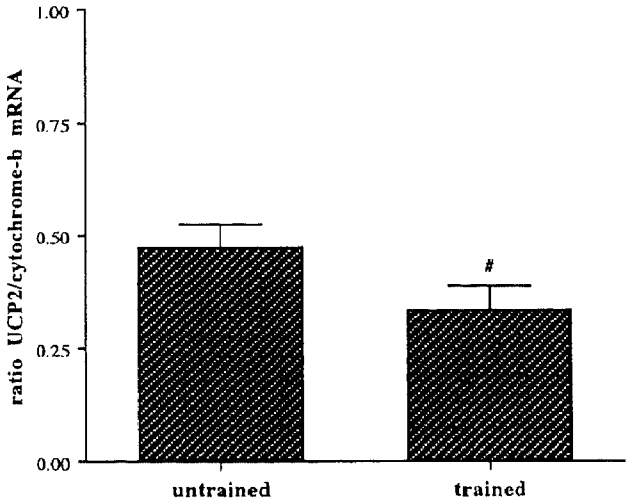
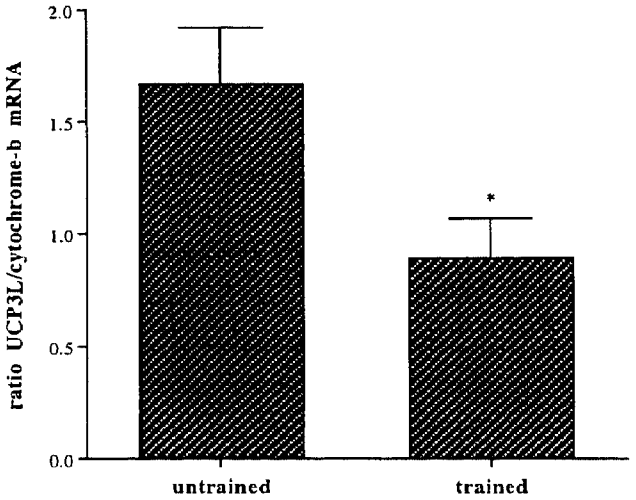


Figure 1 The effect of acute exercise on skeletal muscle *UCP2, UCP3L* and *UCP3S* expression in six trained subjects.





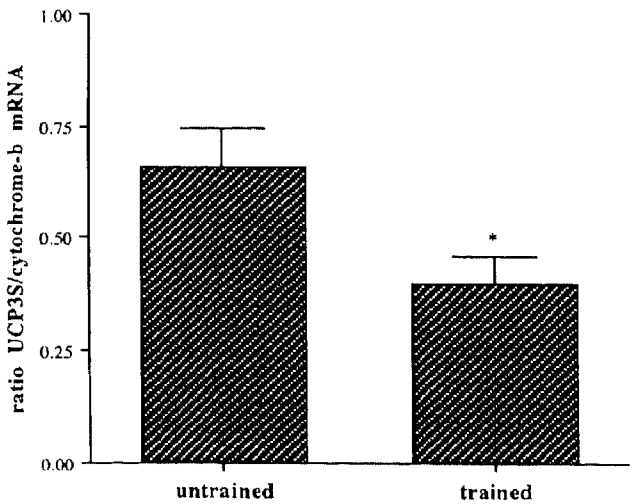


Figure 2 Expression of *UCP2, UCP3L* and *UCP3S* relative to cytochrome-b in skeletal muscle of trained and untrained subjects in the resting state. Values are mean ± s.e.m. $^*P < 0.05$, # $P = 0.09$.

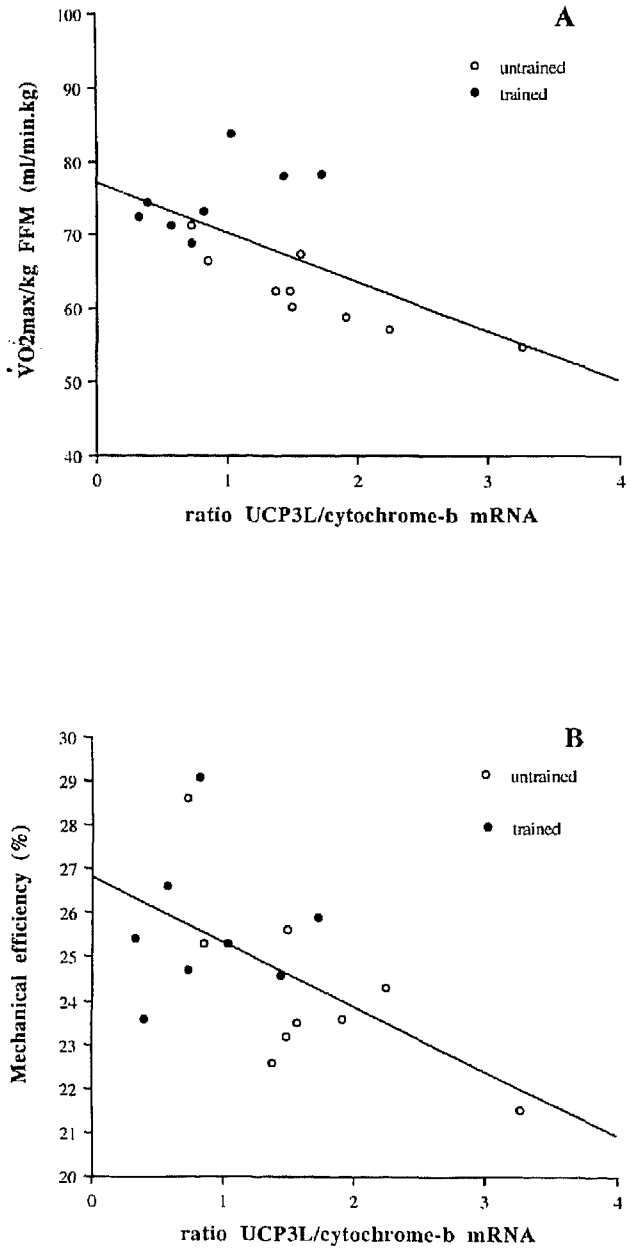


Figure 3 Relationship between A, $\dot{V}O_{2max}$ expressed per kg fat free mass (FFM) and B, mechanical efficiency and UCP3L mRNA expression relative to cytochrome-b (UCP3L/cytochrome-b) in skeletal muscle of trained and untrained subjects. A $\dot{V}O_{2max}$ ($r = -0.61$, $P = 0.009$). B Mechanical efficiency ($r = -0.56$, $P = 0.019$).

Resting respiratory quotient correlated positively with UCP2 mRNA levels ($r = 0.52$, $P = 0.033$). There were no differences in mean plasma concentrations of insulin, T3, leptin and FFA between trained and untrained subjects. The concentrations of the measured hormones did not correlate with the expression of UCP3L, UCP3S or UCP2.

Discussion

The recent discovery of two new uncoupling proteins, UCP2 and UCP3, has renewed interest in the study of human energy metabolism. Both UCP2 and UCP3 have been shown to uncouple respiration from ATP synthesis[2,6] and are therefore likely candidates to underlie some of the variability in energy expenditure and energy efficiency in humans. In this study we found a negative correlation between mechanical efficiency and the expression of UCP3L and UCP3S relative to cytochrome-b, indicating that UCP3 may underlie some of the variability in energy efficiency in humans.

The acute effect of exercise on UCP2/3 expression was measured in six trained subjects. We did not find a decrease in UCP3 immediately after a bout of exercise to exhaustion. The expression of UCP2, however, tended to decrease in response to exercise. Biopsies were taken immediately after cessation of exercise and thus can be considered to be representative for the mRNA expression during the exercise. In this regard, a decrease in UCP2 would allow for a greater energy efficiency during exercise. However, more data are needed to establish the role of UCPs in the regulation of energy efficiency during exercise.

To study the effect of endurance training on UCP expression, we compared levels of UCP2 and UCP3 expression in trained and untrained subjects. The expression of UCP3L, UCP3S and UCP2 mRNA was not different between groups. However, an important metabolic adaptation to endurance training is an increase in mitochondrial content.[24] Since UCPs are mitochondrial proteins a similar expression of UCP mRNA in trained and untrained subjects suggests that the expression of UCPs per mitochondria is decreased in trained subjects. We therefore expressed the mRNA levels of UCP2/3 relative to the mitochondrial mRNA level of cytochrome-b. It has previously been shown that mitochondrial mRNAs for enzymes involved in energy metabolism are increased in direct proportion to the increase in mitochondrial content in response to training,[23] which allows us to use cytochrome-b mRNA as an indicator of mitochondrial content. When expressed relative to cytochrome-b, UCP3L and UCP3S expression was significantly lower in trained compared to untrained subjects. Thus in trained subjects the expression of UCP3L and UCP3S per mitochondria is decreased. This potentially means that there is a less degree of uncoupling between oxidative phosporylation and respiration in the trained subjects, allowing for a higher energy efficiency. Interestingly, we found negative correlations between maximal performance/maximal oxygen consumption and UCP3L expression per mitochondria. This correlation was independent of cytochrome-b expression. Thus, the expression of UCP3L per mitochondria seems to play a role in determining maximal performance and mechanical efficiency. The results indicate that endurance training and/or physical fitness, in addition to cardiovascular, hormonal and metabolic adaptations, reduces the expression of UCP3 per mitochondria, allowing for higher energy efficiency in trained subjects.

It can be suggested that the negative correlation between UCP3 relative to cytochrome-b and W_{max}, $\dot{V}O_{2max}$ or mechanical efficiency was due to the correlation between cytochrome-b and W_{max}, $\dot{V}O_{2max}$ or mechanical efficiency. However, no correlation between cytochrome-b and mechanical efficiency was observed. Furthermore, the negative correlations observed between UCP3L and W_{max}, $\dot{V}O_{2max}$ and metabolic efficiency persisted when only the untrained subjects were considered ($r=0.86$, $P=0.028$). In this sub-group, no correlation between cytochrome-b and W_{max}, $\dot{V}O_{2max}$ or mechanical efficiency were found. Finally, multiple regression analysis revealed that the correlations between UCP3L and W_{max} or $\dot{V}O_{2max}$ were independent of cytochrome-b expression. These results suggest that not only the amount of mitochondria, but also the expression of UCP3 per mitochondria is involved in determining mechanical effiency and maximal performance/oxygen uptake.

As stated above, the negative correlations observed between UCP3L and $\dot{V}O_{2max}$ persisted when only the untrained subjects were considered. Even more, when only the trained subjects were considered the correlation between UCP3L and $\dot{V}O_{2max}$ did not hold true (figure 3a). However, this was mainly caused by three out of the eight trained subjects. Therefore, we can not deduce from our data whether the correlation between UCP3L and $\dot{V}O_{2max}$ is different in trained compared to untrained subjects.

Our finding that the expression of UCP3 relative to cytochrome-b decreases with training is consistent with an increased energy efficiency in trained subjects. This is in support with the finding that gross energy efficiency increases in response to training.[17,18] Also, a decreased thermic effect of food in trained compared to untrained subjects has been found.[25,12] Furthermore, after cessation of training a rapid increase in body weight has been shown, even when food intake is not altered, indicating an increased feeding efficiency.[26]

Regular physical activity has been prescribed in the prevention of obesity. Apart from the increased energy expenditure related to exercise, suggestions have been made that exercise can increase resting metabolic rate. Indeed, endurance training increases resting metabolic rate by increasing fat-free mass. Whether endurance training influences resting metabolic rate after adjustment for body composition is still controversial.[11,12,16] Recently we showed a positive correlation between UCP3 mRNA and resting metabolic rate, adjusted for fat-free mass and fat mass, in Pima Indians.[10] Therefore we would expect training to decrease metabolic rate and not increase it. Taken together, these results do not support the hypothesis that exercise training increases resting metabolic rate after adjustment for body composition.

Conclusions

The decreased levels of UCP3 mRNA per mitochondria in trained subjects and the inverse relationship of this UCP3L expression and mechanical efficiency suggests that exercise training might produce an adaptive physiological response in skeletal muscle improving energy efficiency.

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